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Evaluation of the Analgesic, Anti-inflammatory and Anti-haemorrhoidal Activities of *Àgbo jẹ̀dí.*

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ABSTRACT

Herbal medicines find patronage among both rural and urban populaces. Despite the popularity of these herbal remedies, claims of their therapeutic value remain without validation. *Àgbo jẹ̀dí* (AJ) is an herbal mixture often marketed for the treatment of lower back pain and haemorrhoids. Preliminary phytochemical testing was carried out using standard protocol. Acute toxicity test was done using the OECD No 423 guidelines. Analgesic and anti-inflammatory studies were performed using acetic acid-induced abdominal writhing and formalin-induced paw licking and paw oedema in mice. The effects of AJ on croton oil-induced haemorrhoids in Wistar rats was also tested. Qualitative phytochemical analysis showed presence of tannins, saponins, terpenes, steroids, flavonoids and anthraquinone glycosides. Elemental analysis was positive for K (25.95) > Mg (12.51) > Na (12.04) > Ca (4.01) > Zn (0.3805) ele. No abnormal signs of toxicity or lethality were observed at 20 ml/kg. AJ significantly reduced acetic acid-induced pain by 49.64, 47.45 and 64.23 % at 1.2, 2.4 and 4.8 ml/kg respectively, and diclofenac (10 mg/kg) reduced pain by 88.32%. *Àgbo jẹ̀dí* also exerted 66.67%, 66.67% and 73.33% reduction of formalin-induced pain at 55 - 60 min post pain induction while diclofenac was 88.00%. Paw volume was decreased by 31.5%, 53.93%, 50.79% at 1.2, 2.4 and 4.8 ml/kg doses respectively. Similarly, diclofenac produced 69.84%. Histological assessment showed mild protective actions by AJ as the intensity of damage to recto-anal tissues were not significantly altered. In conclusion, *in-vivo* acute toxicity test carried out showed *'àgbo jẹ̀dí'* to be relatively safe on acute oral administration. It was observed to exhibit analgesic and anti-inflammatory activity but devoid of substantial anti-haemorrhoidal actions.

Keywords: Herbal concoction; *Àgbo jẹ̀dí*; pain; inflammation; haemorrhoids

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INTRODUCTION

Plants have been a fundamental component of healthcare systems since time immemorial and are used in many traditional recipes for the management of a wide range of disease conditions[1,2]. In Nigeria, the use of herbal medicine has been a dominant component of the informal healthcare system with patronage in both rural and urban settings[3]. Some of the plant materials that form the major constituents of these recipes have been scientifically evaluated and data is available that provide information on the pharmacological effects of these herbal medicines, validating their folkloric use as therapies for illnesses[4]. The process of authenticating claims on plants employed as herbal medicines includes identification, documentation of cultivation, conservation and utilization of medicinal plants as well as validation of the therapeutic claims through scientific investigations[5].

Àgbo is a common traditional medicinal product that is prominent among people living in South-Western Nigeria[6]. It is an herbal concoction made from plant parts that may include leaves, stems, roots, bulbs, rhizomes, tubers, seeds, essential oils, gums, exudates, and nectars of plants that are claimed to possess medicinal properties. *Àgbo jẹ̀dí* is prepared, marketed, and used as a remedy for a range of illnesses that include helminthiasis, pain, fever, malaria, stomach disturbances, diabetes, and haemorrhoids (pile)[7,8].

Several studies have been conducted, validating pharmacological actions of herbal medicines that confer therapeutic value in the management of chronic diseases especially. Analgesic, anti-pyretic, anti-plasmodial, anti-spasmodic, anti-diabetic, and anti-heamorrhoidal activities have been reported[7,8]. Additionally, recent studies have demonstrated the anti-arthritic[9], antimicrobial[10], antiepileptic[11], antiviral[12], and anti hypertensive[13] properties of medicinal herbal plants found in Nigeria, validating their use for the management of these conditions.

There are different blends of *Àgbo* produced as infusions or decoctions, using either alcohol or water, depending on the recipe[14]. The production of herbal products does not typically follow stringent guidelines. Hence, compositions vary among traders and across geographical areas[15]. Hence, findings of pharmacological validation studies using herbal medicines produced and commercially distributed in a part of the country are not generalisable and may not hold true for products produced in other parts of the country. This phenomenon introduces a layer of complexity to herbal medicines research and pharmacological validation.

Despite the widespread use of this herbal concoction for management of low back pain and haemorrhoids among people living within the Federal Capital Territory (FCT), these claims have not been sufficiently scientifically validated. This study was therefore designed to investigate the effects of an *Àgbo jẹ̀dí* recipe sold at a major

motor park in Idu-Karmo, Abuja on laboratory models of pain and haemorhoids.

MATERIALS AND METHOD

Herbal samples

The herbal medicinal product, *Àgbo jẹ̀dí* (AJ) was obtained from a commercial source (herbal seller) in the Karmo motor park, Idu-Karmo, Abuja. Nigeria. The product was stored at 4^{o}C before use. Based on the results of the toxicity studies conducted, doses of the product for the acetic acid – induced writhing, formalin – induced paw licking and oedema and antihaemorrhoidal tests was obtained by applying the Human-Equivalent Dosing formula[16] to the adult dosing instructions given by the herbal medicine vendors. In order to administer AJ in a constant volume over the range of doses to be tested, 3 concentrations of AJ were prepared by dilution in distilled water such that graded doses of 1.2 ml/kg, 2.4 ml/kg, and 4.8 ml/kg were delivered in equivalent volumes of 10 ml/kg body weight. Doses were prepared shortly prior to administration.

Animals

Swiss albino mice and Wistar rats of either sex were obtained from the Animal Facility Center of the Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD). The animals were maintained under ambient environmental conditions with free access to rodent diet and water. The animals were housed in plastic lab mice cages with iron-mesh coverings and saw-dust flooring and were acclimatized to laboratory conditions for 72 hours before studies. Experiments were done per the stipulations of the National Institute of Health's guide for the care and use of laboratory animals as documented in the NIPRD SOP document of the Department of Pharmacology and Toxicology NIPRD/05-03-001 to 05-03-004.

Phytochemical screening

Preliminary phytochemical and element content serve as marker components standards for subsequent analysis and for comparison with other *Àgbo jẹ̀dí* blends. Qualitative phytochemical screening of AJ was done using methods described in literature. Tests for saponins, tannins, flavonoids, terpenes, steroids, anthraquinone derivatives, carbohydrates, alkaloids etc were conducted[17,18].

Saponins: AJ (5 ml) was mixed with 20 ml of distilled water and subsequently agitated for 15 minutes. Foaming indicates the presence of saponins[19].

Tannins: AJ (2 ml) was mixed with a few drops of 1% lead acetate solution in a conical flask, with the appearance of a yellowish precipitate indicating the presence of tannins[20].

Terpenes: Salkowski test was conducted. AJ (2ml) was mixed with 2ml chloroform, after which 3ml concentrated sulfuric acid (conc. H_2SO_4) was added. The appearance of a reddish-

brown ring at the interphase demonstrates the presence of terpenes and terpenoids[21].

Flavonoids: The ammonium test was conducted. A small amount of AJ was heated for three minutes in boiling water with 10 ml ethyl acetate. After the cooled mixture was filtered, 1 ml of 1% ammonia solution was mixed with the filtrate and allowed to sit. The presence of flavonoids is confirmed by the appearance of yellow colouration in the ammonia layer[21].

Steroids: A volume of conc. H_2SO_4 was introduced by the test tube's sides after 1 ml of the extract had been dissolved in 10 ml of chloroform. Green fluorescence of the sulfuric acid layer (which subsequently turns yellow) and a red colouration of the upper layer of the solution suggested the presence of steroids[22].

Anthraquinones: Free anthraquinones was tested for by adding 1ml AJ to 10ml chloroform and filtered. The filtrate was then shaken with equal volume of 10% ammonia. A bright pink colouration confirmed the presence of anthraquinones[23].

Carbohydrates: The Fehling's test was conducted where a few drops of AJ were boiled with equal parts Fehling's reagents A and B. Appearance of a brick red precipitate demonstrates the presence of carbohydrates[21].

Alkaloids: The Mayer's test for alkaloids was conducted. A few drops of AJ were warmed with H_2SO_4 after which drops of Mayer's reagents were added and a white precipitation demonstrated the presence of alkaloids[21].

Elemental Analysis

The mineral elements in AJ was brought into solution by digestion as described by Tarfa et al.,2022. From the dried extract 0.5g was weighed and transferred into digestion flask and a mixture of acids (nitric acid (HNO_3): perchloric acid ($HClO_4$) ratio 7:3) was added and left overnight at room temperature. The mixture heated carefully on a powered electrically connected sand-bath in fume hood chamber with periodic addition of digestion mixture until the production of red nitrous oxide (NO_2) ceased and the clear sample solution was obtained cooled and filtered using Whatman No. 42 filter paper into a 50 mL volumetric and made up to mark with deionised water. This was transferred into a capped labelled plastic bottle and kept in refrigerator prior to analysis. A Blank sample and standard (BDH) solutions was also prepared under the same experimental conditions used for the sample preparation without sample analyte. Standard solutions of the metals of interest (the sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), manganese (Mn) and zinc (Zn) were prepared by pipetting out accurately 10 mL of the pure metal, dissolved in concentrated nitric acid (10 mL) and made up to the mark in 100 mL volumetric flask using deionized water). Appropriate concentrations of working standards were prepared from the stock solutions by dilution to volumes in 100 mL volumetric flask with deionized water and transferred into capped plastic bottle prior to absorbance measurement

using Flame Atomic Absorption Spectroscopy FAAS (ThermoFisher Scientific,) after equipment calibration with reference standard (BDH) solutions and blank sample.

The content sample AJ was analysed based on the equipment operating conditions stated in Table 1, after calibration with reference standard solution of the selected elements. The data generated were processed using relation:

$$\text{Metal (mg100/g)} = \frac{\text{C x V x df}}{\text{W(g)}}$$

Where; C is the concentration obtained from the AAS machine (mg/L); V is the volume of the undiluted sample solutions in mL; df is the dilution factor, W is the sample's weight in grams and the result converted.

Table 1: Equipment Operating Conditions and Calibration Data

Element	Wavelength	Flame type	Slit width	Conc. Range (mg/L	R2
Na	589.0nm	Air-acetylene	0.5	0.5-10	0.976
K	766.5nm	Air-acetylene	0.5	0.5-8.00	0.987
Ca	422.7nm	Air-acetylene	0.5	1.5-20.00	0.952
Mg	202.6nm	Air-acetylene	0.5	2.5-20.00	0.997
Mn	279.5nm	Air-acetylene	0.2	0.525-20.00	0.967
Zn	213.9nm	Air-acetylene	0.5	0.525-8.00	0.956

Acute Toxicity test

Acute toxicity testing was done following test No 423 of the Organization for Economic Co-operation and Development (OECD) guidelines[24]. Six female mice were placed into two groups (n = 3). The mice fasted overnight with access to drinking water before the test. Mice in a group received undiluted AJ *per os* at a dose of 20 ml/kg, while mice in the other group which served as control received distilled water. The mice were continuously observed closely during the first 4 h and periodically for 24 h. They were subsequently monitored over 14 days. Signs of toxicity and mortality were observed and recorded.

Acetic acid-induced writhing test

The analgesic activity of AJ was studied using the acetic acid-induced abdominal writhing test[25]. Thirty (30) mice were randomized into five groups of six mice per group. Group 1 served as negative control and received distilled water (10 ml/kg) orally. Groups 2 - 4 received 1.2 ml/kg, 2.4 ml/kg, and 4.8 ml/kg of AJ orally, while group 5 served as positive control and received diclofenac (10 mg/kg). One hour after AJ administration, each mouse was administered 0.65% v/v acetic acid (10 ml/kg) intraperitoneally and transferred to flat surfaces for observation. Abdominal writhing was recorded for each

mouse for a period of 15 min starting 5 min after acetic acid injection. The animals were observed by trained persons who were blind to the treatment given to the animals.

Formalin-induced acute paw licking and oedema test

Thirty mice were randomly placed into five groups of six animals each. *Àgbo jẹ̀dí* was orally administered at 1.2 ml/kg, 2.4 ml/kg, and 4.8 ml/kg to groups 2 - 4 while groups 1 and 5, serving as negative and positive controls, received distilled water (10 ml/kg) and diclofenac (10 mg/kg) respectively. One hour after treatment, 0.05 ml of 2.5% v/v formalin was injected into the sub-plantar tissue of the left hind paw of each mouse. The behavioral signs of pain were subsequently scored following the scoring methods of Dubuisson and Dennis,[26] as follows:

0 – regular weight-bearing on the injected paw

1 – light resting on the paw on the floor

2 – elevation of the injected paw

3 – licking, biting, or grooming of the injected paw.

These responses were observed and recorded for a total of 60 min. The first 10 min (0 - 10 min; scored at 2 min intervals) was considered the early phase, representing aphasic pain while the period between 15 and 60 min (graded every 5 min) was regarded as the late phase representing tonic phase. The most intense reaction observed at every interval was recorded as the pain score. The animals were observed persons who were blind to the treatment given to the animals. After the pain responses were recorded, a digital plethysmometer (LE 7500, Letica Scientific Instruments) was used to record the paw volume. A baseline paw volume was taken before injection of formalin. The paw volume was measured at 1 h, 2 h, 3 h, 4h and 5h after injection with formalin. The difference between paw volume measured after induction and the baseline paw volumes of each mouse was computed as a measure of paw oedema.

Croton oil-induced haemorrhoidal study

The haemorrhoid-inducing agent was prepared with 6 % croton oil, deionized water, pyridine, and diethyl ether in proportion, 10:1:4:5. Wistar rats were weighed and randomized into four groups (A-D) of five rats each. Haemorrhoids were then induced in the animals by insertion of cotton buds impregnated with 0.16 mL of the inducing agent into the ano-rectal region at 20 mm from the anal opening. Contact time of 10 seconds was allowed. Treatment was commenced 24 h after haemorrhoid induction by oral administration of *Àgbo jẹ̀dí*. Group A served as negative control and received distilled water (10 ml/kg), while groups B, C, and D received 1.2 ml/kg, 2.4 ml/kg, and 4.8 ml/kg, respectively. Treatment was administered at 24 hour-intervals over ten days. On Day 10, 24 h after the last treatment, animals were weighed and euthanized by diethyl ether inhalation. The distal 20 mm of anorectal tissue from the anal opening was harvested, weighed, and preserved in 10%

buffered formalin for histopathologic studies[27]. The anorectal coefficient was calculated as follows:

$$\text{Anorectal coefficient} = \left(\frac{Weight\ of\ harvested\ anorectal\ tissue\ (g)}{Total\ body\ weight\ on\ day\ 10\ (g)} \times 100\right)\%$$

An illustration of the timeline of experiments is presented in figure 1.

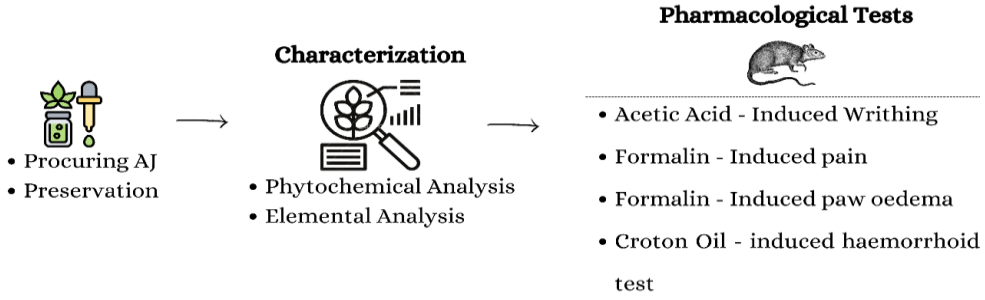

Figure 1. *Timeline of experiments.*

Statistical analysis

Results are presented as mean ± SEM. Statistical comparison was made by analysis of variance (ANOVA) with the data further subjected to various post hoc tests for multiple comparisons. Statistical analysis was carried out using GraphPad Prism version 6. Differences between means were accepted as significant at p values ≤ 0.05.

RESULTS

Phytochemical analysis

Preliminary qualitative phytochemical analysis of *Àgbo jẹ̀dí* showed the presence of tannins, saponins, terpenes, steroids, flavonoids, anthraquinone glycosides and carbohydrates.

Elemental analysis

Some of the macro elements (Ca, Mg, Na, K) and trace elements (Mn and Zn) detected in the tested sample are shown in Table 2. The detected mineral elements are present in decreasing order as K (25.95) > Mg (12.51) > Na (12.04) > Ca (4.01) > Zn (0.3805) mg/100g dry sample.

Table 2. The mineral elements in mg/100g

Element	*Àgbo jẹ̀dí* (…)	FAO/WHO /Limit (mg)	RDA mg/day
Sodium (Na)	12.04 ± 0.05	-	407 - 2092
Potassium (K)	25.95 ± 0.03	0.02	200 - 1300
Calcium (Ca)	4.01 ± 0.01	2000	1000 - 1200
Magnesium (Mg)	12.51 ± 0.01	-	30 - 420
Manganese (Mn)	0.13 ± 0.01	0.01	1.8 - 2.3
Zinc (Zn)	0.38 ± 0.31	0.05	2 - 13

Acute toxicity testing

No mortality or signs of toxicity were observed at the limit dose used (20 ml/kg). No gross changes in behavior, physical activity, and appearance were produced all through the observation period.

Effect of *Àgbo jẹ̀dí* (AJ) on acetic acid induced abdominal writhing

Intraperitoneal administration of acetic acid elicited a pain response in the mice characterized by abdominal contortions (writhes). Pretreatment of animals with AJ caused inhibition of the abdominal contortions. AJ at doses of 1.2, 2.4 and 4.8 ml/kg inhibited abdominal writhing by 49.64%, 47.45% and 64.23% respectively while the reference agent diclofenac caused an inhibition of 88.32%. Although the effect was not dose-dependent, statistical significance ($p<0.05$) was established with the test agents on comparison with the negative control group (Table 1).

Table 3: Effect of *Àgbo jẹ̀dí* (AJ) on acetic acid-induced writhing in mice

Treatment	Number of writhing	% inhibition
Control 10 ml/kg	22.83 ± 11.39	-
AJ 1.2 ml/kg	11.50 ± 3.73*	49.64
AJ 2.4 ml/kg	12.00 ± 1.67*	47.45
AJ 4.8 ml/kg	8.17 ± 6.31*	64.23
Diclofenac 10 mg/kg	2.67 ± 1.21*	88.32

Data expressed as Data ± SEM, n = 6, *$p \leq 0.05$, One-way ANOVA followed by post hoc Dunnett's multiple comparison test vs negative control

Effect *Àgbo jẹ̀dí* on formalin-induced pain in mice

Injection of formaldehyde solution into the sub-planter region of the mice left hind paws elicited pain responses including shaking, licking, biting, grooming or favouring the injected paw. Administration of AJ caused inhibition of the parameters under observation. The reduction in the pain score with corresponding percentage inhibition are reflected in table 2. Significant ($p \leq 0.05$) reduction in the pain score was recorded at 55 - 60 min for 1.2 ml/kg and 4.8 ml/kg, while 2.4 ml exhibited earlier onset of pain score reduction starting from 45- 50 min when compared to control group. The effect of diclofenac was significant from 25 - 30 min post formalin injection.

Effect *Àgbo jẹ̀dí* on formalin-induced paw oedema

Intra-plantar injection of formalin produced an increase in paw volume. Pre-treatment with AJ caused a decrease in the paw volume which was significant at 5h post formalin injection. A statistically significant inhibition of paw oedema corresponding to 53.97% and 50.79% was observed at 2.4, 4.8 ml/kg, with diclofenac exhibiting 63.49 % paw oedema inhibition. Results of the formalin-induced pain and paw oedema tests conducted are presented in tables 4 and 5.

Table 4: Effect of *Àgbo jẹ̀dí* (AJ) on formalin-induced pain in mice

	Control		**1.2 ml/kg**		**2.4 ml/kg**		**4.8 ml/kg**		**Diclofenac 10 (mg/kg)**	
	Phase 1 (Early phase)									
Time (min)	Score	% Inhibition	Score	% Inhibition	Score	% Inhibition	Score	% Inhibition	Score	% inhibition
0-2	3.00 ± 0.00	-	3.00 ± 0.00	0.00	3.00 ± 0.00	0.00	2.67 ± 0.21	11.11	2.50 ± 0.22	16.67
2-4	2.33 ± 0.49	-	1.83 ± 0.31	21.43	2.33 ± 0.21	0.00	1.33 ± 0.21	42.86	1.33 ± 0.21	42.86
4-6	2.00 ± 0.45	-	0.33 ± 0.21[a]	83.33	0.83 ± 0.40	58.33	2.17 ± 0.40	-8.33	1.00 ± 0.45	50.00
6-8	1.67 ± 0.49	-	1.00 ± 0.52	40.00	1.67 ± 0.56	0.00	1.00 ± 0.36	40.00	1.00 ± 0.36	40.00
8-10	1.67 ± 0.56	-	1.17 ± 0.47	30.00	0.50 ± 0.50	70.00	1.50 ± 0.43	10.00	1.33 ± 0.33	20.00
	Phase 2 (Late phase)									
15-20	2.67 ± 0.21	-	2.00 ± 0.63	25.00	2.67 ± 0.33[a]	0.00	1.17 ± 0.54	56.25	1.67 ± 0.42	37.5
20-25	2.83 ± 0.17	-	2.50 ± 0.50	11.76	2.67 ± 0.33	5.88	2.50 ± 0.22	11.76	1.50 ± 0.22	47.06
25-30	3.00 ± 0.00	-	2.50 ± 0.50	16.67	2.50 ± 0.50	16.67	2.83 ± 0.17	5.55	0.83 ± 0.40[b]	72.33
30-35	2.83 ± 1.67	-	3.00 ± 0.00	-5.88	2.33 ± 0.49	17.65	2.17 ± 0.54	23.53	1.00 ± 0.25[b]	64.71
35-40	2.83 ± 0.17	-	2.00 ± 0.63	29.41	2.00 ± 0.51	29.41	2.00 ± 0.45	29.41	1.16 ± 0.31[a]	58.71
40-45	2.67 ± 0.33	-	1.50 ± 0.50	43.75	1.33 ± 0.49	50.00	2.17 ± 0.31	18.75	1.00 ± 0.25[a]	62.50
45-50	2.33 ± 0.49	-	1.33 ± 0.49	42.86	0.83 ± 0.54[a]	64.29	2.17 ± 0.54	7.14	0.83 ± 0.31[a]	64.43
50-55	2.33 ± 0.49	-	1.33 ± 0.61	42.86	0.83 ± 0.54[a]	64.29	1.00 ± 0.45	57.14	0.67 ± 0.33[a]	71.43
55-60	2.50 ± 0.34	-	0.83 ± 0.48[a]	66.67	0.83 ± 0.54[a]	66.67	0.67 ± 0.33[b]	73.33	0.50 ± 0.22[b]	80.00

Data expressed as Data ± SEM, n = 6, [a]$p \leq 0.05$, [b]$p \leq 0.01$, Two-way ANOVA followed by post hoc Dunnett's multiple comparison test vs negative control

Table 5: Effect of *Àgbo jẹ̀dí* (AJ) on formalin induced paw oedema in mice

Time (h)	Control		1.2 ml/kg		2.4 ml/kg		4.8 ml/kg		Diclofenac	
	Vol	% Inhibition	Vol	% Inhibition	Vol	% Inhibition	Vol	% Inhibition	Vol	% Inhibition
0	0.073 ± 0.012	-	0.076 ± 0.007	-	0.077 ± 0.007	-	0.078 ± 0.010	-	0.075 ± 0.007	-
2	0.085 ± 0.013	-	0.072 ± 0.008	15.69	0.072 ± 0.016	15.69	0.068 ± 0.009	19.61	0.062 ± 0.08	27.45
3	0.12 ± 0.012	-	0.095 ± 0.012	17.39	0.093 ± 0.012	18.84	0.078 ± 0.015	31.88	0.062 ± 0.01[a]	55.07
4	0.11 ± 0.020	-	0.085 ± 0.011	21.54	0.077 ± 0.009	29.23	0.068 ± 0.015	36.92	0.030 ± 0.005[c]	61.29
5	0.11 ± 0.012	-	0.072 ± 0.009	31.75	0.048 ± 0.019[b]	53.97	0.052 ± 0.007[b]	50.79	0.038 ± 0.011[c]	69.84

Data expressed as Data ± SEM, n = 6, [a]$p \leq 0.05$, [b]$p \leq 0.01$, Two-way ANOVA followed by Dunnett's multiple comparison test vs negative control

Effect of *Àgbo jẹ̀dí* (AJ) on croton-oil-induced haemorrhoids in rats

Histological examination showed anal application of croton oil caused severe damage to recto-anal tissues. Infiltration of inflammatory cells, spots of haemorrhage, villi necrosis, adhesion and dilation of blood vessels were observed. Animals treated with AJ presented with moderate infiltration of inflammatory cells and moderate villi adhesion. Significant effect on inflammation was observed at 4.8 ml/kg while the effect on necrosis was not statistically significant (Table 4). Dose-dependent effects were not clearly observed between the cellular architecture of the recto-anal tissues of treatment groups and control (Figure 2).

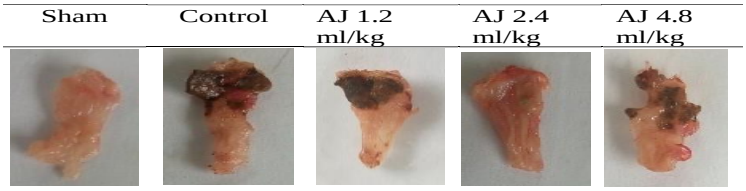

Figure 2. Effect of *Àgbo jẹ̀di* (AJ) on croton oil induced haemorrhoids

Representative images showing the effect of *Àgbo jẹ̀dí* on croton oil induced haemorrhoids on rat recto-anal tissues

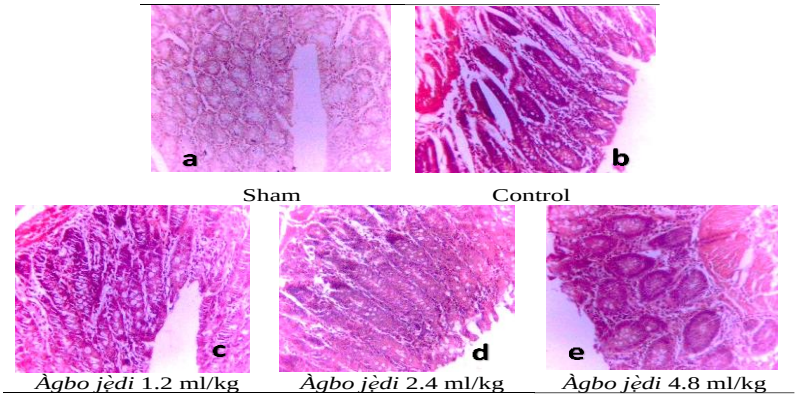

Figure 3: Effect of *Àgbo jẹ̀dí* on recto anal tissues in croton oil induced haemorrhoids

(a) sham group showing normal features (b) negative control showing hyperplasia of inflammatory cells, degeneration, villi necrosis, and hemorrhage, (c) *Àgbo jẹ̀dí* treated group showing moderate hyperplasia of inflammatory cells, necrosis (d) Villi adhesion and moderate infiltration of inflammatory cells (e) moderate infiltration of inflammatory cells, moderate villi necrosis

Table 6. Histology of anal tissues for effect of *Àgbo jẹ̀di* on croton oil induced haemorrhoids

	Histology	
Dose (ml/kg)	**Inflammation**	**Necrosis**
Sham	0.28 ± 0.06	0.22 ± 0.03
Control	2.82 ± 0.06	2.82 ± 0.06
AJ 1.2	2.68 ± 0.06	2.74 ± 0.06
AJ 2.4	2.62 ± 0.06	2.60 ± 0.05
AJ 4.8	2.50 ± 0.09[a]	2.62 ± 0.09

Values are expressed as mean ± SEM; n = 6; One-way ANOVA [a]p<0.05 treatment vs negative control.
Score results are taken based on observations from 10 fields of histology slides.
The scores for features examined: normal = 0, slight = 1, moderate = 2, severe = 3.

Table 7. Anorectal coefficients of animals in AJ - treated vs control groups

Dose (ml/kg)	**Anorectal Coefficient (%)**
Control	0.2432 ± 0.0002
AJ 1.2	0.3558 ± 0.0016
AJ 2.4	0.3057 ± 0.0010
AJ 4.8	0.2450 ± 0.0007

Data presented as mean ± SEM; [a] - $p \leq 0.05$; Two-way ANOVA followed by Dunnett's multiple comparison test vs negative control

Discussion

The effects of *Àgbo jẹ̀dí* (AJ) on pain, inflammation and haemorrhoids were investigated using animal models. The effects on pain was investigated using acetic acid-induced pain. Formalin was used to induced pain and inflammation, while activity on haemorrhoids were evaluated using croton oil induced haemorrhoids. In this study AJ demonstrated analgesic and anti-inflammatory actions.

The acetic acid writhing test is a widely used model to evaluate analgesic and anti-inflammatory effects of test agent[28]. This test assesses the peripheral analgesic property of test substances[29]. The injection of irritant chemicals such as dilute acetic acid into the peritoneum of mice causes secretion of endogenous mediators of pain such as prostaglandins and leukotrienes[30]. The release of these substances stimulates pain nociceptors,[31] thereby resulting in pain which is expressed by movements characterized by abdominal constrictions (writhing) in the animals. Analgesic action is deduced as decrease in the number of abdominal writhes[29]. In this study, administration of *Àgbo jẹ̀dí* exhibited

analgesic action by significant reduction of induced abdominal writhes. The mechanism of action by which the constituents AJ demonstrated analgesic action may not be completely explained from the data of this study, but from other studies, it has been proposed that plant extracts demonstrate analgesic effects by inhibition of the release of pain mediators, or act by interaction with pain receptors in the peripheral nervous system to reduce the pain sensation caused by acetic acid[32].

Formalin-induced pain tests for effects of substances on pain and inflammation. Intra-plantar administration of dilute formalin caused pain response characterized by shaking, biting, licking or favoring of the injected paw. The pain response is scored according to the intensity or duration of the behavioural response. Potential analgesic agents would generally decrease the score of the pain response or reduce the amount of time the animals spend grooming the injected paw. In the formalin test, the early phase (central) results essentially from the direct stimulation of nociceptors, whereas the late phase (peripheral) involves a period of sensitization during which inflammatory reactions occur[33,34]. As propounded by Tjolsen and colleagues,[33] the early phase of formalin-induced nociception which lasts for 3 – 5 minutes is mediated primarily by direct stimulation of nociceptors by formalin. Subsequently, a period of little to no signs of nociception lasts for 10 – 15 minutes and about 15 – 20 minutes after administration of the pain-inducing agent, the second phase of nociception commences, lasts for up to 40 minutes and is characterized by a peripheral inflammatory process, as well as a cascade of spinal processes precipitated by the first phase. Fundamentally and patho-physiologically, both phases differ beyond the intensity of pain experienced. Signalling molecules; bradykinin and substance p appear to be involved in the first phase while the second phase is mediated by histamine, serotonin, bradykinin, and prostaglandins[33]. Treatment of animals with AJ produced analgesic effect evidenced by pain scores which were reduced in treated animals compared to the untreated control group. The pain-relieving effect was observed to be significant in the second phase therefore indicating that *Àgbo jẹ̀dí* was more effective against peripherally mediated pain as indicated from the data obtained. Findings of the acetic acid – induced writhing and formalin – induced pain and paw licking tests are consistent with earlier studies that have demonstrated the pharmacological activities of herbal extracts to inhibit pain and inflammation using similar pharmacological models[35,36].

Haemorrhoids refer to pathological changes and distal displacement of haemorrhoidal tissues which are vascular cushions surrounding the distal rectum and anal canal that help maintain anal continence[37]. The condition is often characterized by vasodilation and inflammation in the rectal region, resulting in increased vascular permeability and extravasation of inflammatory cytokines in the interstitial

space[38]. Common initial symptoms include pain, rectal bleeding, discomfort, rectal itch, swelling and protrusion of rectal blood vessels[39]. Croton oil causes hemorrhoids by development of vasodilation, oedema and inflammatory processes.[40] It has been proposed that inflammation is the main mechanism responsible for the damage of the connective tissues around the anus, ischaemia, thrombosis and ulceration of hemorrhoidal tissues. Croton oil activates inflammatory pathways extravasations followed by release of inflammatory mediators like prostaglandins, kinins, cytokines, leukotrienes, and nitric acid at the site of cellular injury[38,41,42]. In this study, *Àgbo jẹ̀dí* exhibited anti-inflammatory actions by causing a decrease in mouse paw volume of formalin-induced oedema.

Histological evaluation of the rectal tissues showed severe to moderate degree of infiltration of inflammatory cells, villi necrosis, adhesion of rectal tissues and haemorrhage caused by administration of the phylogistic agent. The damaging effect of this substance on the integrity of rectal tissues was not overall mitigated by treatment with *Àgbo jẹ̀dí* because necrosis and venous dilation were still observed in treatment groups despite the significant anti-inflammation seen at 4.8 ml.kg. The restorative effect of *Àgbo jẹ̀dí* on recto-anal tissues is slight/mild in this study suggesting that AJ may not be very potent in causing healing of the haemorrhoidal tissues. It could also be that the duration of treatment was not adequate to produce effective venotonic, venoprotective and restorative actions by *Àgbo jẹ̀dí*. However, the goal of medical treatment of haemorrhoids is to control acute symptoms (e.g. pain and inflammation) especially in non-advanced and uncomplicated disease, rather than achieve a cure of the hemorrhoids[43]. Therefore, the popularity of the use of *Àgbo jẹ̀dí* for haemorrhoids could be attributed to its effects in alleviating the symptoms of the condition. Preliminary phytochemical analysis has revealed the presence of phytochemicals that include tannins, saponins, terpenes, triterpenes, flavonoids, anthraquinone glycosides. In other studies, these plant biochemical compounds have demonstrated analgesic and inflammatory actions[44]. Thus, suggesting that the constituents of the cocktail *Àgbo jẹ̀dí* may have inhibitory effects against the release of inflammatory markers[45]. These compounds have also been shown to demonstrate anti-haemorrhoidal actions in previous studies[2,38,46].

Minerals are essential chemical elements required for various physiological processes that are necessary for the normal functioning of the organism. Macro elements which include calcium, phosphorus magnesium, sodium, potassium, sulphur and chloride are required in relatively large quantities. They are involved in functions that include maintaining fluid balance, nerve and muscle functions. While, trace minerals such as iron, zinc, selenium, manganese etc are needed in much small amounts[47,48]. Trace elements (present in minute amount but

necessary for life) are required for functions that include immune regulation, nerve conduction, muscle contraction, membrane potential regulation, mitochondrial activity and enzyme reactions[49] Although the role of macro elements and trace elements in hemorrhoids have not been well elucidated, minerals such as magnesium, calcium, manganese, zinc have shown anti-inflammatory activities in animal and human studies by inhibition of inflammatory mediators[50,51]. Magnesium is proposed to play a protective effect for chronic pain in neurological disorders[52]. Other studies suggest that magnesium is functional as an adjunct for pain management as it has no direct analgesic effect. Magnesium inhibits calcium ions entering cells by blocking NMDA receptors, which causes an antinociceptive effect. Furthermore, this antinociceptive effect is related to its prevention of central sensitization caused by peripheral tissue injury[53,54]. The macro elements and some trace element detected in this sample of *Àgbo jẹ̀dí* include Na, K, Mg and Ca while some of the trace elements that were present in the sample are Zn, Mn. Thus, the minerals detected may be playing a contributary role in the analgesic and anti-inflammatory function of *Àgbo jẹ̀dí* in the management of haemorrhoids.

Acute toxicity tests indicate that *Àgbo jẹ̀dí* is relatively safe on acute oral administration as no mortality or abnormal behavioural pattern was recorded during the observation period[24]. However, there are concerns about safety especially with chronic use of *Àgbo jẹ̀dí*. Akinboro *et al*[7] reported possible cytotoxic effects. Due to the chronic nature of haemorrhoidal conditions and the consequent need for repeated doses of AJ in their treatment, repeated dose toxicity studies to further elucidate the safety/toxicity profile of *Àgbo jẹ̀dí* are under way.

Conclusion

The data obtained showed that the herbal preparation demonstrated potential analgesic, anti-inflammatory and mild protective effects against croton oil induced haemorrhoids. The ethnomedical application of *Àgbo jẹ̀dí* for pain and haemorrhoids can be attributed to its analgesic and anti-inflammatory which can be attributed to the presence of secondary plant metabolites that include saponins, terpenes, steroids, flavonoid detected in the herbal preparation. *Àgbo jẹ̀dí* is relatively safe on oral acute administration.

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Conflict of Interest

The Authors declare no conflict of interest.

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